REMARKS

In view of the above amendments and following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version with Markings to Show Changes Made.

The improvement of plant growth by the application of organic fertilizers has been known and carried out for centuries. Modern man has developed a complex inorganic fertilizer production system to produce an easy product that growers and farmers can apply to soils or growing crops to improve performance by way of growth enhancement. Plant size, coloration, maturation, and yield may all be improved by the application of fertilizer products. Inorganic fertilizers include such commonly applied chemicals as ammonium nitrate. Organic fertilizers may include animal manures and composted lawn debris, among many other sources.

In most recent years, researchers have sought to improve plant growth through the use of biological products. Insect and disease control agents such as *Beauveria bassiana* and *Trichoderma harizamum* have been registered for the control of insect and disease problems and thereby indirectly improve plant growth and performance.

There is some indication of direct plant growth enhancement by way of microbial application or microbial by-products. Nodulating bacteria have been added to seeds of leguminous crops when introduced to a new site. These bacteria may improve the nodulation efficiency of the plant and thereby improve the plant's ability to convert free nitrogen into a usable form, a process called nitrogen fixation. Non-leguminous crops do not, as a rule, benefit from such treatment. Added bacteria such as *Rhizobium* directly parasitize the root hairs, then begin a mutualistic relationship by providing benefit to the plant while receiving protection and sustenance.

Mycorrhizal fungi have also been recognized as necessary microorganisms for optional growth of many crops, especially conifers in nutrient-depleted soils. Mechanisms including biosynthesis of plant hormones, increased uptake of minerals, and water have been postulated. Mycorrhizal fungi have not achieved the common frequency of use that nodulating bacteria have due to variable and inconsistent results with any given mycorrhizal strain and the difficulty of study of the organisms.

Plant growth-promoting rhizobacteria ("PGPR") have been recognized in recent years for improving plant growth and development. Hypothetical mechanisms range from direct influences (e.g., increased nutrient uptake) to indirect mechanisms (e.g., pathogen displacement). Growth enhancement by application of a PGPR generally refers to inoculation with a live bacterium to the root system and achieving improved growth through bacterium-produced hormonal effects, siderophores, or by prevention of disease through antibiotic production, or competition. In all of the above cases, the result is affected through root colonization, sometimes through the application of seed coatings. There is limited information to suggest that some PGPR strains may be direct growth promoters that enhance root elongation under gnotobiotic conditions; however, the production of plant growth regulators has been proposed as the mechanism mediating these effects. Many bacteria produce various plant growth regulators *in vitro* or antibiotics. Siderphore production is another mechanism proposed for some PGPR strains. The colonization of root surfaces and thus the direct competition with pathogenic bacteria on the surfaces is another mechanism of action. Canola (rapeseed) studies have indicated PGPR increased plant growth parameters including yields, seedling emergence and vigor, early-season plant growth (number of leaves and length of main runner), and leaf area. Studies with potato indicated greater yields when *Pseudomonas* strains were applied to seed potatoes. Yield increase was apparently due to the competitive effects of the PGPR to eliminate pathogenic bacteria on the seed tuber, possibly by antibiosis. In several studies, plant emergence was improved using PGPR. Numerous other studies indicated improved plant health upon treatment with rhizobacteria, due to biocontrol of plant pathogens.

Pathogen-induced immunization of a plant has been found to promote growth. Injection of *Peronospora tabacina* externally to tobacco xylem not only alleviated stunting but also promoted growth and development. Immunized tobacco plants, in both greenhouse and field experiments, were approximately 40% taller, had a 40% increase in dry weight, a 30% increase in fresh weight, and 4-6 more leaves than control plants. These plants flowered approximately 2-3 weeks earlier than control plants.

The present invention is directed to an improvement over prior plant growth enhancement procedures.

The finality of the written restriction requirement is respectfully traversed. A Petition Under 37 C.F.R. § 1.144 For Review of Restriction Requirement accompanies this amendment.

The objection to the drawings is respectfully traversed in view of applicants' submission of the accompanying formal drawings.

The rejection of claims 38-39, 41, and 46-50 under 35 U.S.C. § 112 (1st para.) for lack of written description in the specification is respectfully traversed.

The basis for this rejection is that the present application does not describe any DNA molecules encoding an *Erwinia amylovora* hypersensitive response elicitor other than that having the nucleotide sequence of SEQ. ID. No. 4. The outstanding office action further notes that there is no disclosure of the *hrpW*, *dspE*, and *dspF* genes from this species. However, patent applications for these genes and the proteins they encode were filed on long after the filing date of the present application. In any event, as demonstrated *infra*, hypersensitive response elicitors and the genes encoding them are an art-recognized class of compounds and, therefore, achievement of the results shown in the examples of the present application with particular hypersensitive response elicitors would be expected for other hypersensitive response elicitors.

In plants, the hypersensitive response phenomena results from an incompatible interaction between plant pathogens and their non-host plants. As explained in Gopalan et al., "Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis," Plant Disease 80: 604-10 (1996) ("Gopalan") (attached hereto as Exhibit 1), this interaction involves a bacterium attempting to infect a host plant, preventing multiplication and spreading of the pathogen, and collapse of plant leaf cells and cell death at the site of infection. This is distinct from a compatible interaction between bacteria and a plant where the bacteria spread in the infected plant, leading to disease symptoms throughout the plant. Id. at 604.

Genes controlling hypersensitive response elicitation and pathogenesis (i.e., *hrp* genes) are recognized in the art to be present in a limited class of Gram-negative pathogens, including *Erwinia, Pseudomonas, Burkholderia,* and *Xanthamonas* pathogens. See Bonas, "Bacterial Home Goal by Harpins," Trends Microbiol. 2: 1-2 (1994) ("Bonas I") (attached hereto as Exhibit 2); Bogdanove et al., "Unified Nomenclature for Broadly Conserved *hrp* Genes of Phytopathogenic Bacteria," Molec. Microbiol. 20:681-83 (1996)

("Bogdanove") (attached hereto as Exhibit 3); and Gopalan. These genes are found in large clusters of 20-40 kb and are conserved physically and functionally amongst different species. Bonas I, Bogdanove, and Gopalan.

Hypersensitive response elicitors have a number of common characteristics. These include their being glycine rich, heat stable, hydrophilic, capable of inducing a hypersensitive response in tobacco after recombinant expression, susceptible to proteolysis, and cysteine lacking. See Bonas, "*hrp* Genes of Phytopathogneic Bacteria," Current Topics in Microbiology and Immunology 192: 79-98 (1994) ("Bonas II") (attached hereto as Exhibit 4); Bonas I; and Preston et al., "The HrpZ Proteins of *Pseudomonas syringae* pvs. *syringae, glycinea,* and *tomato* are Encoded by an Operon Containing *Yersinia ysc* Homologs and Elicit the Hypersensitive Response in Tomato but not Soybean," MPMI 8(5): 717-32 (1995) ("Preston") (attached hereto as Exhibit 5).

The hypersensitive response elicitors within a given genus are also homologous to other elicitors from different pathogenic species of that genus. For example, *Erwinia amylovora* harpin is homologous to other *Erwinia* spp. harpins. See Willis et al., "*hrp* Genes of Phytopathogenic Bacteria," MPMI 4(2): 132-38 (1991) ("Willis") (attached hereto as Exhibit 6); Ahmad et al., "Harpin Is Not Necessary for the Pathogenicity of Maize," 8th Int'l Cong. Molec. Plant Microbe Inter. July 14-19, 1996 ("Ahmad I") (attached hereto as Exhibit 7); and Gopalan. In addition, *Pseudomonas syringae* pv. *syringae* harpin is homologous to other *Pseudomonas syringae* harpins. See Gopalan; Preston; and Willis. Different *Pseudomonas solancearum* harpins have also been found to be homologous to one another. Arlat et al., "PopA1, a Protein which Induces a Hypersensitivity-like Response on Specific *Petunia* Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solancearum,*" EMBO J. 13(3): 543-53 (1994) ("Arlat") (attached hereto as Exhibit 8) and Willis.

Further, genes encoding hypersensitive response elicitors from different genera have been found to be similar. For example, the *Xanthamonas campestris* and *Pseudomonas solancearum* harpin encoding genes are similar to one another, while those from *Erwinia amylovora* and *Pseudomonas syringae* are also similar. Van Gijsegem et al., "Conservation of Secretion Pathways for Pathogenicity Determinants of Plant and Animal Bacteria," Trends Microbiol. 1: 175-80 (1993) ("Van Gijsegem") (attached hereto as Exhibit 9) and Bogdanove.

In Bauer et al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995) ("Bauer et al. 1995") (attached hereto as Exhibit 10), the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene coding for the *Erwinia chrysanthemi* hypersensitive response elicitor as follows:

> The cosmids were probed in colony blots with a 1.3-kb *Hind*III fragment from pCPP1084, which contains the *E. amylovora hrpN* gene (Wei et al. [, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (]1992[)]). pCPP2157, one of the three cosmids hybridizing with the probe, was digested with several restriction enzymes, and the location of the *hrpN$_{Ech}$* gene in those fragments was determined by probing a Southern blot with *E. amylovora Hind*III fragment. Two fragments, each containing the entire *hrpN$_{Ech}$* gene, were subcloned into different vectors: pCPP2142 contained an 8.3-kb *Sal*I fragment in pUC119 (Vieira and Messing [,"Production of Single-Stranded Plasmid DNA," Methods Enzymol., 153:3-11(] 1987[)]), and pCPP2141 contained a 3.1-kb *Pst*I fragment in pBluescript II SK(-) (Stratagene, La Jolla, CA).
>
> ***Sequence of hrpN$_{Ech}$***
>
> The nucleotide sequence of a 2.4-kb region of pCPP2141 encompassing *hrpN$_{Ech}$* was determined. The portion of that sequence extending from the putative ribosome-binding site through the *hrpN$_{Ech}$* coding sequence to a putative rho-independent terminator is presented in Figure 1.

See page 485. As noted in Bauer et al., "*Erwinia chrysanthemi hrp* Genes and Their Involvement in Soft Rot Pathogenesis and Elicitation of the Hypersensitive Response," MPMI 7(5): 573-81 (1994) ("Bauer et al. 1994") (attached hereto as Exhibit 11), a probe carrying a fragment of the *Erwinia amylovora* hypersensitive response elicitor encoding gene not only hybridizes to the gene encoding the hypersensitive response elicitor for *Erwinia chrysanthemi* but also to the gene encoding the hypersensitive response elicitor for *Pseudomonas syringae* p.v. *syringae*. See abstract and pages 574 and 576. In addition, Cui et al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N$_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996) ("Cui") (attached hereto as Exhibit 12) further indicates

that the gene encoding the *Erwinia carotovora* hypersensitive response elicitor can be isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora* in the same manner as that probing gene was found from the *Erwinia amylovora* hypersensitive response elicitor encoding gene, as discussed *supra.* Further, Cui (at page 572) reads as follows:

> The genomic library of *E. carotovora* subsp. *carotovora* strain Ecc71 in pLARF5 was screened by in situ colony hybridization with a 0.75-kb internal *Cla*I fragment of *hrpN* of *E. chrysanthemi* (Bauer et al. 1995). Two cosmids, pAKC921 and pAKC922, that hybridized with the probe were isolated. The subclones (pAKC923 and pAKC924, Table 1) carrying *hrpN* DNA were used for sequence analysis.

The gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia stewartii*. It was additionally found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora*. See Ahmad I and Ahmad et al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996 ("Ahmad II") (attached hereto as Exhibit 13).

Further, the hybridization studies in Laby et al., "Hybridization and Functional Complementation of the *hrp* Gene Cluster from *Erwinia amylovora* Strain Ea321 with DNA of Other Bacteria," Mol. Plant-Microb. Inter. 5: 412-19 (1992) ("Laby") (attached hereto as Exhibit 14) indicate that probes from the hypersensitive response elicitor encoding gene cluster for *Erwinia amylovora* hybridized to genomic DNA from other *Erwinia* species and from *Pseudomonas syringae* species.

From all of the foregoing literature, there is ample support to show that hypersensitive response elicitors from plant pathogens are a well known phenomenon caused by a limited number of plant pathogens. In view of the similarity amongst the hypersensitive response eliciting proteins of different pathogenic species, as well as the similarity of the genes encoding the various hypersensitive response elicitors, these elicitors clearly constitute an art recognized class of compounds, at least with regard to their method of use. Accordingly, one of ordinary skill in the art would expect that the results shown in the examples of the present application with particular hypersensitive response elicitors would be

expected to be achieved with other hypersensitive response elicitors, including other hypersensitive response elicitors from *Erwinia amylovora*. Accordingly, the rejection under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written description requirement should be withdrawn.

The rejection of claims 38-39, 41, and 46-50 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed.

Page 26, line 21 through page 31, line 2 and page 32, line 28 through page 34, line 2 of the present application outline the basic steps involved in the generation of transgenic plants including recombinant DNA tools and techniques, as well as the transfection of plants with transformation constructs. The techniques were well established and known in the art prior to filing of the present application.

The ability of transgenic plants transformed with nucleic acid molecules encoding hypersensitive response elicitors to enhance growth of such plants, taught by the present application, is confirmed by the accompanying Declaration of Zhong-Min Wei under 37 C.F.R. § 1.132 ("Wei Declaration").

In confirming this phenomena, Arabidopsis plants were transformed with either: (1) DNA constructs containing the *hrpN* gene inserted between a NOS promoter and a NOS terminator or (2) using a transformation construct identical to that of (1) except that a *pr1b* signal sequence was inserted between the NOS promoter and the *hrpN* hypersensitive response elicitor encoding gene from *Erwinia amylovora* (Wei Declaration ¶ 6). Both sets of *hrpN* transgenic Arabidopsis plants, with and without the signal sequence, showed increased growth of approximately 23% over non-transgenic Arabidopsis plants (Wei Declaration ¶ 7).

Cotton transformed with the *hrpN* hypersensitive response encoding elicitor from *Erwinia amylovora* was also produced and tested for growth enhancement (Wei Declaration ¶8). Assays conducted comparing the non-transgenic and transgenic cotton plants showed growth enhancement of the transgenic cotton of approximately 10-28% over the growth of the non-transgenic plants (Id.).

From the results of the Wei Declaration, it is thus clear that plants or plant seeds transformed with a nucleic acid molecule encoding a hypersensitive response elicitor from *Erwinia amylovora* achieve enhanced growth.

Additionally, as described in paragraphs 10-11 of the Wei Declaration, topical treatment of plants with hypersensitive response elicitors originating from *Psuedomonas*

syringae (i.e., HrpZ) and *Xanthomonas campestris* (i.e., HreX), are also capable of enhancing the growth.

A partially purified HreX hypersensitive response elicitor protein was used to treat tomato seed prior to sowing, and the resulting plants were evaluated with respect to plant height (Wei Declaration ¶ 10). HreX treated plants grew substantially more than buffer-treated control plants (Id.).

In a similar study, tomato seeds were treated with the partially purified HrpZ hypersensitive response elicitor protein, and the resulting plants were analyzed for growth enhancement (Wei Declaration ¶ 11). Treatment of tomato seeds with HrpZ resulted in tomato plants that grew substantially more than the buffer-treated control plants (Id.). Example 24 of the present application provides further evidence that treatment of plants with a hypersensitive response elicitor from *Psuedomonas syringae* enhances plant growth.

It is thus clear that hypersensitive response elicitors from a range of sources have the ability to enhance plant growth.

Thus it is clearly evident from the present application, the Wei Declaration, and the above remarks that: (1) plants and plant seeds transformed with the HrpN elicitor from *Erwinia amylovora* achieve enhanced growth; (2) plants topically treated with hypersensitive response elicitors from *Erwinia amylovora, Pseudomonas syringae*, and *Xanthomonas campestris* achieve enhanced plant growth; and (3) hypersensitive response elicitors are an art-recognized class of compounds with common biochemical characteristics and biological functions. Accordingly, one of ordinary skill in the art would expect the results in the Wei Declaration, showing that transgenic plants encoding a hypersensitive response elicitor from *Erwinia amylovora* exhibit enhance growth, to be replicated in transgenic plants encoding other hypersensitive response elicitors.

Finally, it is understood by those skilled in the art that many variables may affect a plant's response after transformation with a recombinant DNA molecule. Factors such as strong or weak constitutive promoter, inducible promoters, and lethality of recombinant DNA expression are routinely taken into account.

Since the claimed invention is fully enabled by the present application, the non-enablement rejection under 35 U.S.C. § 112 (1st para.) should be withdrawn.

The rejection of claims 38-39, 41, and 46-50 under 35 U.S.C. § 112 (2nd para.) as indefinite, is respectfully traversed in view of the above amendments and the following remarks.

With regard to claim 38 and the language "conditions effective to enhance plant growth," applicants refer to page 32, line 28 through page 34, line 2, and pages 34-82 (i.e., Examples 1-24) of the present application. These passages provide a clear description of how plant growth is enhanced in accordance with the present invention. Furthermore, claim 38 is clear with regard to what is done with the plant and plant seed of line 3. In particular, either plants themselves (if they are provided) are grown or plants produced from the seeds (if the seeds are what is provided) are grown.

Accordingly, the rejection of claims 38-39, 41, and 46-50 under 35 U.S.C. § 112 (2nd para.) should be withdrawn.

The rejection of claims 38-39, 41, and 46-50 under the judicially created doctrine of obviousness-type double patenting over claims 15-16 of U.S. Patent No. 6,174,717 to Beer et al. ("Beer") is respectfully traversed.

The claims in the present application are patentable over claims 15-16 of Beer, because, unlike the claims of the present application, the claims of Beer relate to transgenic plants generally and have nothing to do with a method of growth enhancement in plants. Since a method for enhancing the growth of plants would not have been obvious from the claims of Beer, the rejection over that reference should be withdrawn.

The rejection of claims 38-39, 41, and 46-50 under the judicially created doctrine of obviousness-type double patenting over claims 11-16 of U.S. Patent No. 6,228,644 to Bogdanove et al. ("Bogdanove") is respectfully traversed.

The claims in the present application are patentable over claims 11-16 of Bogdanove, because, unlike the claims of the present application, the claims of Bogdanove relate to transgenic plants generally and have nothing to do with a method of growth enhancement in plants. Since a method for enhancing the growth of plants would not have been obvious from the claims of Bogdanove, the rejection over that reference should be withdrawn.

In view of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Date 9/27/2002 JoAnn Whalen

Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made to claims 38, 39, and 41, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In the Claims:

38. (Amended) A method of enhancing growth in plants compared to untransformed plants or plant seeds comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and

growing the transgenic plants or transgenic plants produced from the transgenic plant seeds under conditions effective to enhance plant growth.

39. A method according to claim 38, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas,* and *Phytophthora*[, and mixtures thereof].

41. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein is [corresponds to that] derived from *Erwinia amylovora.*